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BIO-ASSAY INVESTIGATIONS FOR INTERNATIONAL JOINT COMMISSION. NIAGARA FALLS SEWAGE DISPOSAL PLANT, NIAGARA FALLS, NEW YORK

Croswell Henderson

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1956

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FOR

INTERNATIONAL JOINT COMMISSION

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Robert A. Taft Sanitary Engineering Center

Cincinnati, Ohio

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BIO-ASSAY INVESTIGATIONS

NIAGARA FALLS SEWAGE DISPOSAL PLANT

NIAGARA FALLS, NEW YORK

PREPARED BY

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This report covers an investigation of the toxicity to fish of the influent and effluent from the Niagara Falls Sewage Disposal Plant.

Chemical wastes from several industries enter this plant along with domestic sewage from the city of Niagara Falls, New York. Chemical wastes may contain compounds which are highly toxic to aquatic life. These compounds, when mixed with or under the influence of other non-toxic components of the effluent or receiving waters, may exert an entirely different toxicity from that of the pure compounds. Bio-assays were made to evaluate directly the toxicity of these chemically complex wastes which enter the Niagara River.

Organization of Study

Bio-assays were made on samples collected during Februray and March 1956. Sewage plant personnel collected 24 hour composite samples. Public Health Service personnel performed bio-assays in laboratory space provided by National Aniline Division - Allied Chemical and Dye Corporation in Buffalo. Analyses for phenols and cyanides were made in the Buffalo I. J. C. Unit Laboratory.

Bio-Assay Methods

Bio-assays were made essentially by the method recommended by the Toxicity Subcommittee of the Federation of Sewage and Industrial Wastes Associations (Sewage and Industrial Wastes, Vol. 23, No. 11, 1380, Nov. 1951). This method consists of preparing various concentrations of effluent in a selected dilution water, adding the test fish and observing their reactions over a definite time period. For ease in plotting and interpreting results it is desirable to make up test solutions with the percentage of effluent in a logarithmic series as 10, 5.6, 3.2, 1.8, 1.0 etc.

As the effluents were of unknown toxicity, exploratory or small scale tests were made to determine the approximate toxic range. Test solutions were prepared over a wide range of concentration (e.g., 100, 10, and 1.0 per cent effluent). Two fish were added to 2 liters of each concentration in 6-1/2 inch diameter, 1 gallon widemouth glass bottles. Observations for relatively short time periods indicated test concentrations necessary for the full scale experiments.

In full scale bio-assays, 10 fish are normally used for each test concentration. Five fish are added to 10 liter duplicate samples in 5 gallon widemouth glass bottles, 10 inches in diameter. In these tests, however, duplicates were not always used. Some of the bio-assay results are based on a series of concentrations containing 5 fish in 10 liters. The intermediate concentrations tested were dependent upon information obtained from the exploratory tests. For example, if fish were killed in concentrations above 10 per cent and not affected in concentrations of 1

per cent, intermediates were set up within this range (e.g., 10, 5.6, 3.2, 1.8, and 1.0 per cent concentration of effluent).

The dilution water used was raw Lake Erie water obtained at the Buffalo, New York, water plant. This water was hauled into the laboratory, allowed to come to room temperature, and aerated vigorously for at least one hour to bring it to equilibrium with atmospheric gases. Characteristics of this water at time of use were as follows: Dissolved oxygen 7.6-8.2 ppm; pH 8.0-8.2; total alkalinity ($CaCO_3$) 94-100 ppm; total acidity ($CaCO_3$) 0-1 ppm; versenate hardness ($CaCO_3$) 120-135 ppm.

The test fish used were fathead minnows (Pimephales promelas), ranging in length from 2 to 2-1/2 inches and in weight from 1 to 1-1/2 grams. These fish were obtained in uniform lots from the Newtown, Ohio, Fish Hatchery and acclimated to laboratory conditions. This species is of intermediate tolerance to chemicals comparable to bass, sunfish, perch, and other warm water species, and will tolerate fairly low oxygen conditions (1-2 ppm).

The bio-assays were made at ordinary laboratory temperatures which were within the range from 22 to 25° C. and compare favorably with maximum summer water temperatures in this area.

The tests are designed so that generally no oxygenation or aeration is needed. Absorption of atmospheric oxygen by the exposed water surface is normally adequate for fish requirements during the test period. However, in some bio-assays, high oxygen demand effluents caused oxygen depletion. When necessary, dissolved oxygen was maintained by bubbling pure oxygen through the test solution by means of a suitable arrangement of

valves and small tubing. The rate (60-180 bubbles per minute) was adjusted to maintain adequate oxygen for fish survival with minimum agitation in order to prevent the loss of volatile materials.

Physical and chemical determinations (temperature, dissolved oxygen, pH, alkalinity, and hardness) were made on each concentration initially, after fish mortality, or at the completion of the test. This was done primarily to detect low D.O. levels so they could be controlled and to differentiate between fish mortality due to oxygen deficiency or acidity and toxic properties.

Effluents were normally brought into the laboratory in the afternoon immediately following collection and exploratory tests were set up at once. Observations made the following morning furnished information on the toxicity of the wastes essential for setting up the full scale tests.

Fish reactions were observed over a 96 hour period. From the mortality in different concentrations, 24, 48, and 96 hour TL_m (Median tolerance limit) values were obtained. The median tolerance limit is the concentration of effluent in dilution water that kills just 50 per cent of the test fish. These values were obtained by straight line graphical interpolation from points representing per cent survival of fish and log concentrations of effluents which bracketed the 50 per cent point.

The 96 hour TL_m was used to compute the dilution ratio, which is the ratio of a unit volume of effluent to dilution water which produces a 50 per cent mortality of the test fish. This ratio times the effluent flow gives the dilution volume or the total flow or volume of receiving water required to reduce the toxicity of the effluent to where a 50 per

cent mortality of the test fish is produced. The following formula may be used for directly computing this dilution volume:

$$\frac{100 - TL_m}{TL_m} \times \text{Effluent Flow} = \text{Dilution Volume}$$

The dilution volumes represent conditions which would cause direct injury to fish upon short time exposure and are a direct comparative measure of toxicity. Liberal application factors must be applied to these results for complete protection of aquatic life. These factors are based on the following major considerations:

(1) The bio-assay procedure measures 50 per cent mortality during a relatively short time period in non-renewed solutions. This must be related to concentrations having no effect during continuous long time exposure.

(2) Test data apply directly to the species of fish used. While fathead minnows are of intermediate tolerance and compare favorably with many warm water "game" fishes, some locally important species of fish and fish food organisms may be more sensitive. Bio-assays conducted on various industrial wastes with a locally important forage fish, the emerald shiner (Notropis atherinoides), indicated this species was approximately twice as sensitive as fathead minnows.

(3) Some effluents may vary in toxicity. A few samples may give an indication of toxicity but maximum conditions may be missed. In many situations, maximum conditions of toxicity are the limiting factor as far as aquatic life is concerned.

(4) Some conditions which may generally reduce but in some cases increase

the magnitude of the application factor are the loss of volatiles, hydrolysis, oxidation, precipitation, and changes in water quality characteristics of the receiving water.

Based on the best available information, as much as 10 times the computed dilution volume may be necessary for protection of aquatic life in the receiving water.

For these wastes, it is believed that an even higher application factor may be necessary. The greatly increased toxicity in 96 hours over that for the 24 hour period indicates a chronic or accumulative toxicity. Further information is needed to fully determine what the value of the application factor should be.

Sampling

Hourly grab samples were collected and composited over a 24 hour period. Most of the samples taken were of influent to the disposal plant. Major treatment at the plant is removal of suspended solids, which would not be expected to have an effect on toxicity. One sample, however, of both influent and effluent were taken during the same sampling period for comparison. Ten gallon samples were adequate for the bioassays.

The average flow from this plant into the Niagara River was estimated at 80 mgd.

RESULTS

Physical and Chemical Characteristics

A general description and some of the chemical characteristics of the samples are shown in Table 1.

All samples were similar in general appearance, having a grayish tan

TABLE 1 - PHYSICAL AND CHEMICAL DATA

NIAGARA FALLS SEWAGE TREATMENT PLANT

Source	Date 1956	Description Color, odor, etc.	Dissolved Oxygen ppm	pH	Alkalinity ($CaCO_3$) ppm	Acidity ($CaCO_3$) ppm	Hardness ($CaCO_3$) ppm	Phenols ppm	Cyanide ppm
Sewage Plant Influent 24 hr. composite	2-21	Grayish-tan color Very sour odor Large amount of gray flocculent material	6.7	10.6	370	0	550	1.66	0.07
"	3-15	"	1.0	8.8	160	0	525	0.94	0.06
Effluent	3-15	"	1.8	8.7	150	0	515	0.94	0.06
Influent	3-18	"	6.8	11.7	570	0	810	-	-
"	3-20	"	8.0	10.9	156	0	575	-	-

color, a very sour odor and containing large amounts of a gray flocculent material.

Samples taken on three of the four sampling dates were also quite similar in physical and chemical characteristics. The February sample and two of the March samples were high in dissolved oxygen (6.7-8.0) and were highly alkaline (10.6-11.7). Dissolved oxygen levels were well within limits expected to support aquatic life. For adequate protection of aquatic life, dissolved oxygen concentration should be maintained at 5 ppm or higher in waters receiving toxic materials. Values for pH were somewhat higher than those considered suitable for aquatic life. Generally pH values below 5 and above 9.5 may have a direct lethal effect on fish. Within these values, a change in pH may have an effect by increasing or decreasing the toxicity of chemicals to fish. The toxicity of metals, cyanides, sulfides, etc. may be increased by a decrease in pH while the toxicity of ammonia and other chemicals may be greater at higher pH values. The buffering capacity of the dilution water, however, was such that in the bioassay test concentrations, pH values were at levels which would not be expected to directly cause fish mortality.

The March 15 influent and effluent samples were quite similar in all charactistics. These samples contained less dissolved oxygen (1.0-1.8 ppm) and were also at lower pH values (8.7-8.8) than the others. These were the only samples in which there was an apparent high oxygen demand. It was necessary to add oxygen artificially in some of the bioassay test concentrations to maintain an adequate supply for the test fish. Test concentrations of other samples did not require the addition of oxygen but remained at adequate levels during the test period.

Phenols and cyanides were at levels below those expected to have an

adverse effect on aquatic life.

No residual chlorine was found in any of the samples.

Fish mortality in the bioassays was apparently due to unknown toxic compnents.

Toxicity to Fish

A summary of bioassay results showing the comparative toxicity of the different samples to fish is shown in Table 2. The data on which these results are based are shown in the Appendix tables at the end of this report. Twenty-four, 48, and 96 hour TI_m values and dilution volumes were computed as described in the section on bioassay methods.

All of the samples were toxic to fish. The February sample and two of the March samples were highly toxic with the March 15 samples considerably less toxic. The slightly increased toxicity of the effluent over influent in the March 15 sample could have been caused by low oxygen in one of the test concentrations of effluent.

An interesting observation was that on the same day (March 15) that the sewage plant waste was at the lowest toxicity level, the main sewer effluent from the Hooker Electrochemical Company (one of the wastes which enter the disposal plant) was much less toxic than previously.

The sewage plant samples that were the most toxic had higher dissolved oxygen values and were at higher pH levels than the less toxic sample. Possibly the high toxicity and pH have an effect in reducing biochemical oxygen demand.

These effluents had an apparent chronic toxic effect. Fish mortality was greatly increased between the 24 and 96 hour periods, but in a few tests

TABLE 2 - SUMMARY OF BIOASSAY DATA

NIAGARA FALLS SEWAGE DISPOSAL PLANT EFFLUENTS

Source	Date 1956	Effluent Flow cfs	TL_m (Median Tolerance Limit) (Percent Concentration) 24 hr	48 hr.	96 hr.	Dilution Volume cfs
Influent to Plant	2-21	123	8.2	3.7	3.5	3391
Influent to Plant	3-15	123	24	22	20	492
Effluent from Plant	3-15	123	20	20	15	697
Influent to Plant	3-18	123	13	8.0	4.8	2439
Influent to Plant	3-20	123	13.5	7.5	4.5	2610

allowed to continue for a longer period, mortality was not greatly increased. This effect was somewhat similar to that observed in previous bioassay work when using chlorinated hydrocarbons, such as toxaphene and dieldrin.

A definite physiological reaction of fish was observed from these effluents. The abdomen of the fish began swelling after about 18 hours exposure to low effluent concentrations and were swelled to approximately twice normal size in from 24 to 48 hours. This was followed by a breakdown of tissue beneath the skin, as evidenced by red streaks on the belly and sides. The fish either died or returned to normal in the period from 72 to 96 hours (This swelling had been observed previously in laboratory bioassays with dieldrin. The breakdown of tissue, however, had not been previously observed with this or other chemicals).

CONCLUSIONS

A limited bioassay study of this nature can only give an indication of possible effects on receiving waters. The volume of this effluent is large and some of the samples were highly toxic to fish. Based on the effluent flow and an application factor of 10 (which may not be adequate due to chronic toxicity), approximately 1/5 of the flow of the Niagara River would be necessary for dilution for safety to aquatic life. If it were not for turbulence and rapid mixing in Niagara River at the point of discharge, damage to aquatic life would be evident. To adequately evaluate this situation, further studies would be necessary, including bioassays and field biological studies to determine effects on the Niagara River biota.

APPENDIX TABLE 1 -BIOASSAY DATA

NIAGARA FALLS SEWAGE DISPOSAL PLANT

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Influent to	2-21-56	100	-	-								Influent	25	6.7	10.6	370	0	550	Grayish-tan color.
plant																			Large amount of gray light flocculent suspended material.
24 hr.composite		32	5	10	2 hrs.	3 hrs.	5 hrs.	0	0	0	0	Initial	25	7.4	9.2	100	0	230	Very sour odor
In Lab												5 hrs.	25	6.6	9.0	94	0	225	
5:00 PM 2/21		18	10	20	5 hrs.	5-22 hrs.	5-22 hrs.	0	0	0	0	Initial		7.8	9.1	124	0	210	Fish swelled to about twice normal size in 24 hours.
Fish Added												22 hrs.		5.4	8.7	116	0	220	
11:00 AM 2/22		10	10	20	5-22 hrs	24 hrs.	52 hrs.	40	10	0	0	Initial		7.8	8.8	112	0	180	Breakdown of tissues evidenced by red marks on belly and sides.
												52 hrs.		3.0	8.0	104	2	180	
		5.6	10	20	5-22 hrs	29-46 hrs	29-46 hrs	70	0	0	0	Initial		7.8	8.6	106	0	160	Some recovered in 72-96 hours and returned to normal.
												46 hrs.		4.2	7.7	110	4	155	
		3.2	10	20	24 hrs.	-	-	90	70	70	60	Initial		7.4	8.6	100	0	140	
												96 hrs.		4.6	7.8	114	4	160	
		1.8	10	20	24 hrs.	36 hrs.	-	80	60	60	50	Initial		7.6	8.3	100	0	140	
												96 hrs.		5.0	7.8	106	4	145	
		Control	10	20	-	-	-	100	100	100	100	Initial		7.8	8.1	102	2	130	
												96 hrs.		5.0	7.7	102	4	130	
		TLm (Median Tolerance Limit - Per Cent Concentration)						8.2	3.7	3.7	1.5								
Influent to	3-13-56	100	-	-								Influent	24	1.0	8.8	160	0	525	Light grayish-tan.
plant																			Gray suspended material which settles rapidly to clear solution.
24 hr.composite		32	5	10	4 hrs.	6 hrs.	8 hrs.	0	0	0	0	Initial	24	4.8	8.6	112	0	250	Very sour odor.
In Lab												8 hrs.	24	1.0	8.0	108	6	245	
6:00 AM 3/15		18	5	10	48 hrs.	-	-	100	80	60	60	Initial		6.4	8.5	108	0	200	
Fish Added												96 hrs.		4.8	7.5	116	6	215	No swelling or red marks observed.
2:00 PM 3/17		10	5	10	53-65 hrs	-	-	100	100	80	80	Initial		7.0	8.4	96	0	165	
												96 hrs.		5.0	7.5	104	10	185	
		Control	5	10	-	-	-	100	100	100	100	Initial		7.8	8.3	92	2	115	
												96 hrs.		6.2	7.6	102	6	130	
		TLm (Median Tolerance Limit - Per Cent Concentration)						24	22	20	20								
Affluent from	3-13-56	100	-	-								Effluent	24	1.8	8.7	150	0	515	Same as above.
plant.																			
24 hr.composite		32	5	10	4 hrs.	6 hrs.	8 hrs.	0	0	0	0	Initial	24	5.8	8.5	110	0	250	
In Lab												8 hrs.	24	0.1	8.0	110	8	250	Low oxygen.
6:00 AM 3/15		18	5	10	8-22 hrs.	22-66 hrs.	-	60	60	40	40	Initial		6.6	8.5	100	0	200	
Fish Added									D.O.-3.0			96 hrs.		5.8	7.4	114	12	230	No evidence of swelling or breakdown of tissues.
2:00 PM 3/17		10	5	10	48 hrs.	-	-	100	80	80	80	Initial		7.2	8.4	100	0	165	
												96 hrs.		4.8	7.5	110	12	175	
		TLm (Median Tolerance Limit - Per Cent Concentration)						20	20	15	15								

APPENDIX TABLE 2 -BIOASSAY DATA

NIAGARA FALLS SEWAGE DISPOSAL PLANT

TEST FISH - FATHEAD MINNOWS
DILUTION WATER - LAKE ERIE

SOURCE AND METHOD OF COLLECTION OF SAMPLE	DATE OF COLLECTION	CONCENTRATION TESTED PERCENT	NUMBER OF TEST FISH	VOLUME OF TEST SAMPLE LITERS	TIME OF DEATH			PERCENT SURVIVAL				CHEMICAL AND PHYSICAL DATA							REMARKS
					FIRST FISH	HALF OF FISH	ALL OF FISH	24 HOURS	48 HOURS	72 HOURS	96 HOURS	TIME	TEMP. °C	DISSOLVED OXYGEN ppm.	p H	TOTAL ALK ($CaCO_3$) ppm.	TOTAL ACIDITY ($CaCO_3$) ppm	VERSENATE HARDNESS ppm.	
Influent from	3-18-56	100	-	-								Influent	24	6.8	11.7	570	0	810	Grayish-tan color. Large amount of gray suspended material. Settles to clear solution. Strong sour odor.
plant																			
24 hr.composite		32	5	10	1 hr.	2 hrs.	2 hrs.	0	0	0	0	Initial	24	5.8	10.9	162	0	275	
In Lab												2 hrs.	24	7.0	11.0	120	0	250	
5:00 PM 3/18		18	5	10	2-15 hrs.	2-15 hrs.	2-15 hrs.	0	0	0	0	Initial		7.2	10.2	132	0	145	High pH
Fish Added												15 hrs.		6.6	9.8	94	0	160	High pH
5:00 PM 3/19		10	5	10	2-15 hrs.	24-39 hrs	-	80	20	20	20	Initial		7.4	9.4	96	0	120	Fish began to swell and turn red after 24 hours. Recovered after 72 hours.
												96 hrs.		5.4	8.5	70	0	150	
		5.6	5	10	51 hrs.	66-72 hrs	-	100	100	40	40	Initial		8.0	9.4	116	0	160	
												96 hrs.		6.4	8.1	122	6	165	
		3.2	5	10	48 hrs.	-	-	80	80	80	80	Initial		8.6	8.9	108	0	150	
												96 hrs.		4.8	7.8	114	6	150	
		Control	5	10	-	-	-	100	100	100	100	Initial		7.8	8.3	92	2	115	
												96 hrs.		6.2	7.6	100	6	130	
		TLm (Median Tolerance Limit - Per Cent Concentration)						13	8.0	4.8	4.8								
Influent from	3-20-56	100	-	-								Influent	24	8.0	10.9	156	0	575	Grayish-tan color. Large amount of gray suspended material. Clear when settled. Sour odor.
plant.																			
24 hr.composite		32	2	2	2 hrs.	2 hrs.	3 hrs.	0	0	0	0	Initial	24	-	-	-	-	-	
In Lab												3 hrs.	24	8.4	9.4	134	0	265	
6:00 PM 3/20		18	5	10	6-18 hrs.	6-18 hrs.	24 hrs.	0	0	0	0	Initial		9.0	9.3	112	0	200	
Fish Added												24 hrs.		7.6	9.0	112	0	220	
2:30 PM 3/21		10	5	10	27-42 hrs	27-42 hrs	-	100	40	20	20	Initial		9.0	9.1	102	0	170	Fish started swelling after 18 hours. After 48 hours swelled and red on sides. Some recovered by 96 hrs.
									D.C.-3.6			96 hrs.		5.4	7.8	104	8	180	
		5.6	5	10	27-42 hrs	48-51 hrs	-	100	60	20	20	Initial		9.0	8.8	94	0	140	
									D.O.-4.0			96 hrs.		6.0	7.8	102	6	165	
		3.2	5	10	-	-	-	100	100	100	100	Initial		9.0	8.6	92	0	130	
									D.O.-4.0			96 hrs.		5.2	7.7	102	8	145	
		Control	5	10	-	-	-	100	100	100	100	Initial		9.0	8.1	90	2	120	
												96 hrs.		5.8	7.3	92	6	120	
		TLm (Median Tolerance Limit - Per Cent Concentration)						13.5	7.5	4.5	4.5								